

REMARKS

Claims 1-35 are cancelled. Claims 36, 38-40, 42, 43, 46-50, 52, 53, and 56-58 are amended. Claims 60-73 are withdrawn from consideration by the examiner.

Claims 36-57 and 59 stand rejected under 35 USC 103(a) as being unpatentable over Green et al (US 7,547,817) in view of Qui et al (Intl. Immunol. 1999; 11: 37-46).

Claim 58 stands rejected under 35 USC 103(c) as being unpatentable over Green et al in view of Qui et al and further in view of GenBank AC073553 (September 2002).

Claims 36 and 52 stand rejected under 35 USC 103(a) as being unpatentable over Green et al in view of Qui et al and further in view of Harriman et al.

Each of these rejections are addressed and traversed in the following remarks.

Background of the invention

The IgH locus structure and the mechanism of class switch recombination are well-known in the art (see *Immunology, Ivan Roitt et al., Third Edition, 1993, Mosby, pages 5.9 to 5.11 and 11.9 to 11.12; Annex I*). B cell maturation requires the expression of membrane IgM on B cells surface. The production of a selected antibody isotype requires the development of IgM producing B cells which subsequently undergo isotype class switching from IgM to the IgG or IgA, or IgE isotypes. Isotype class switch is mediated through a deletional recombination event (class switch recombination or CSR) occurring between tandem directly repetitive switch regions (S) present 5' of all IgH constant region genes except Cδ. Transcription starting from an I exon located immediately upstream of the switch region and continuing through the switch region is required for CSR. Enhancers and cytokine response sequences are known to lie in the region near the I promoter.

Brief description of the claimed invention

The claimed invention relates to a transgenic knock-in mammal, wherein the endogenous immunoglobulin heavy chain locus (IgH) has been modified by homologous recombination to replace the switch sequence S μ with a transgene containing a human heavy chain constant region gene C α or a segment of the C α gene containing at least an exon encoding the CH3 domain and a membrane exon.

Figure 1 of the present specification illustrates the endogenous IgH locus in mice, the targeting vector which can be used for homologous recombination and the murine locus after homologous recombination.

The deletion of the switch sequence S μ in the endogenous IgH locus and its replacement with the human C α transgene produces the following effects on the immunoglobulin heavy chain genes expression:

- (i) a chimeric human IgA heavy chain in which the constant region is human and the variable region is from the non-human mammal is expressed to high level; this chimeric IgA heavy chain benefits from a completely diversified repertoire corresponding to the normal repertoire generated by rearrangements of the VH, D and JH segments at the non-human mammal IgH locus,
- (ii) the expression of the endogenous C μ gene responsible for the synthesis of IgM heavy chains is abolished, and
- (iii) the expression of the other immunoglobulin heavy chains genes which code for the other classes of immunoglobulins (IgG and IgE) is greatly reduced due to the blocking of the Ig class switch toward the immunoglobulin constant genes located downstream of C μ on the endogenous IgH locus.

These specific characteristics of the non-human transgenic mammal of the claimed invention are demonstrated in the examples. For example, Example 1 demonstrates that the non-human transgenic mammal of the claimed invention produces antibodies which contain large quantities of chimeric human IgAs (in the gram per litre range in mice) but no IgM. Example 3 demonstrates that the transgenic mammal is capable of producing

antibodies with high affinity as a secondary response to the antigen since its B lymphocytes can recruit the somatic hypermutation phenomenon.

The brief description of the claimed invention above is intended solely for purposes of clarification and is not intended to limit either the scope of any pending claims.

Green et al. (US 7,547,817)

Green describes human antibodies producing transgenic mammals (Xenomouse™) which are obtained by introducing yeast artificial chromosome cloning vectors containing large germline fragments (unrearranged) of the human Ig locus (human Ig YAC transgene), into the somatic and germline cells of a mammal (column 2, line 31 to column 3, line 8 and column 4, lines 4-14).

Green describes that current technologies for obtaining a transgenic mouse which produces an antibody of the desired isotype requires antibody re-engineering *in vitro* which is labor intensive, slow and expensive (column 4, line 60 to column 5, line 36).

Green is thus directed to solving the specific problem of obtaining a pre-selected human antibody isotype from a transgenic mouse (column 5, lines 53-55).

Green describes that due to the differential responsiveness of mouse and human switch regions to lymphokines and other activators it is desirable to have heterologous switch regions controlling CSR in human antibody producing mice (column 12, lines 29-33).

Green discloses a transgenic mammal containing in its somatic and germline cells an unrearranged human immunoglobulin heavy chain (IgH) YAC transgene containing VH genes, all the D elements, all J elements, S μ , C μ and C δ from human chromosome 14, and a chimeric human IgH constant region gene in which human constant region gene sequences encoding the desired heavy chain isotype are operably linked to an

heterologous or non-cognate switch region (column 5, line 66 to column 6, line 3; column 6, lines 21 to 36). For example, a mouse switch region is operably linked to a human gamma, alpha or epsilon constant region coding segment, or a human switch region is operably linked to a human constant region segment, the switch region being from a different isotype than the constant region coding segment (column 6, lines 40-45 and 51-55). The heterologous switch region controls switching from the human IgM to the downstream human Ig of the desired isotype. The transgenic mammal of Green is engineered to produce human IgM and human Ig of the desired isotype (column 5, line 66 to column 6, line 3).

A first transgenic mouse line producing human IgM and IgG2 antibodies (Xenomouse) was previously derived by Green, from the yH1C transgene composed of 66 VH genes, all the D elements, all J elements, C μ and C δ and all regulatory elements in germline configuration, appended in 3' with a 22 kb fragment containing the human C γ 2 gene, including its switch region (S γ 2), and a 4 kb fragment containing the mouse 3' enhancer element (column 9, line 58 to column 10, line 42).

The human IgH YAC transgenes disclosed in Green are engineered by introducing a targeting vector having 5' and 3' flanking homology to yH1C and an appropriate selection marker, into yeast carrying yH1C. Such vector can be recombined *in vivo* in yeast to replace the human S γ 2 and C γ 2 with the mouse S γ 1 functionally linked to a human CH coding sequence (*e.g.* human or the human C γ 1) or the human C γ 2 by another human CH gene, in yeast carrying yH1C (column 12, line 36 to column 13, line 9 and figures 1 to 7). To produce an antibody of the desired isotype, new transgenic Xenomouse are then generated by introducing the recombinants IgH YAC transgenes into mouse ES cells.

Harriman *et al.*, J. Clin. Invest., 1996, 97, 477-485 and Qiu *et al.*, Int. Immunol., 1999, 11, 37-46

These two research papers from Harriman are directed to understanding the process of antibody class switching and their implication in IgA deficiency (abstract and introduction of Harriman *et al.*). For this purpose, an I α knock-out mouse was generated by targeted deletion (Figure 1 of Harriman *et al.*) and used in Qiu *et al.* (figure 1 and page 38, 2nd column, beginning of second paragraph which refers to Harriman). In this I α knock-out mouse, a human HPRT mini-gene driven by the PGK promoter and having a SV40 poly(A) signal replaces the I α exon and proximal promoter located upstream of the switch region S α , in the endogenous IgH constant region gene C α . The knock-out mouse is generated by targeted deletion. Therefore, all the sequences 5' and 3' to the I α exon which include S μ and C μ in 5' and S α in 3', are present in the knock-out mouse IgH locus. Harriman *et al.* teaches that the I α knock-out mouse produces mouse IgM, IgG and IgA at levels equivalent to those of the wild-type (page 480, 2nd column, 2nd paragraph and page 481, 1st column, end of first paragraph). Harriman *et al.* teaches also that the I α exon or transcripts containing the I α exon are not required for IgA class switch but a second signal is required for the induction of IgA class switch (abstract). Qiu *et al.* discloses that a transcript of any sequence which is spliced across the switch region is necessary and maybe sufficient for CSR.

GenBank AC073553.5

GenBank AC073553.5 discloses the sequence of a 187523 bp DNA segment from mouse chromosome 12.

Non-obviousness of claims 36-57, 59 over Green et al. in view of Qiu et al.

The rejection of claims 36-57 and 59 under 35 USC 103(a) over Green *et al.* (US 7,547,817) in view of Qiu *et al.* (Int. Immunol., 1999, 11, 37-46) is respectfully traversed as the cited references do not disclose all the elements of the claimed invention, suggest the combination of elements of the claimed invention, or provide a reasonable expectation of success for the claimed invention.

Green was cited as differing from the present claims in that although Green may provide transgenic mice for producing any desired isotypes of human antibodies including IgA, the exemplified target IgH gene is not Ca (page 6 of the present Office Action).

However this is respectfully untrue. It is worth noting that the Examiner herself acknowledges that it is not true since it is noted on page 8 of the present Office Action that “*the combined teaching of Green in view of Qiu teaches a transgenic mouse with a non-cognitive S region in its genome but not lacking a Sμ*”.

Green differs from the present claims in that Green, at the very least, does not disclose or suggest any of the following features from the claimed invention:

1. Green does not disclose a knock-in mammal generated by homologous recombination at the mammal endogenous IgH locus but a transgenic mammal (Xenomouse™) generated by random insertion of large germline fragments of the human IgH locus, into the somatic and germline cells of a mouse using YAC vectors (Column 5, Line 66 to Column 6, Line 3, Column 6, Lines 36-37; Column 7, lines 44-48; Column 9, Line 58 to Column 10, Line 42).

Therefore, contrary to what is stated by the Examiner on page 6 of the present Office Action, Green does not disclose a targeting vector comprising 5' and 3' mouse flanking sequences for homologous recombination, which is introduced into mouse ES cells.

2. Green does not disclose targeted DNA sequence replacement at the mouse endogenous IgH locus. Green discloses targeted DNA sequence replacement in yeast, on a human IgH YAC transgene comprising unrearranged variable region genes (V, D, J genes) Sμ, Cμ, Cδ, Sγ2, Cγ2, and a 4 kb fragment containing the mouse 3' enhancer element (column 12, line 36 to column 13, line 9, cited by the Examiner on page 5 of the present Office action, and figures 1 to 7).

3. Green does not disclose targeted DNA sequence replacement, wherein the endogenous switch sequence $S\mu$ is replaced with a transgene construct comprising a human heavy chain constant region gene $C\alpha$ or a segment of the $C\alpha$ gene comprising at least an exon encoding the CH3 domain and a membrane exon.

Green discloses targeted DNA sequence replacement, wherein: (i) the human $S\gamma 2$ switch sequence and the human $C\gamma 2$ coding sequence are replaced with the mouse $S\gamma 1$ switch sequence functionally linked to a human CH coding sequence (*e.g.* human or the human $C\gamma 1$), or (ii) the human $C\gamma 2$ coding sequence is replaced by another human CH gene (column 6, lines 40-45 and 51-55; column 12, line 36 to column 13, line 9 (cited by the Examiner on page 5 of the present Office action) and figures 1 to 7)..

Therefore, contrary to what is stated by the Examiner on page 5 of the Office action, Green does not disclose the deletion of the mouse $S\mu$ since Green discloses targeted replacement within a human immunoglobulin heavy chain (IgH) transgene. In addition, the targeted replacement disclosed by Green does not contain the replacement of $S\mu$ but of $C\gamma 2$ alone or together with its switch region $S\gamma 2$.

4. Green does not disclose a human heavy chain constant region (CH) transgene which is not linked to a switch region. Green discloses a human CH gene which is always functionally linked to an heterologous switch region (column 6, Lines 29 to 32 and column 12 cited by the Examiner on page 5 of the present Office Action).
5. Green does not disclose the insertion of a human CH transgene between the intronic enhancer $E\mu$ and the $C\mu$ gene (figure 1 of the present application) but downstream of the $C\mu$ gene (figures 1 to 4 of Green).
6. Green does not disclose a modified IgH locus that is incapable of isotype switching from IgM to the isotype of the downstream human CH transgene. Green discloses a human IgH transgene which is always functionally linked to an heterologous switch

region and capable of isotype switching from the human IgM to the isotype of the downstream human CH gene (column 6, lines 26 to 29 and column 12 cited by the Examiner on page 5 of the present Office Action).

7. Green does not disclose a transgenic mammal which produces no IgM and high level of chimeric human IgAs in which the heavy chains comprise a human immunoglobulin A constant region and a variable region from the non-human mammal. Green discloses a transgenic mammal which produces human IgM and human Ig of the desired isotype.

Qiu was cited as disclosing, on figure 1, a transgenic mouse whose endogenous switch region and C α region was replaced with a human S α and C α (page 6 of the present Office Action). However this is respectfully untrue. As mentioned above in paragraph 3.2.3, Qiu discloses an I α knock-out mouse. In this mouse, the endogenous switch region and C α region are not modified and there is no human S α and C α insertion (figure 1 of Qiu or Harriman which both refer to the same mouse).

One of ordinary skill in the art would have no reason to combine Green with Qiu because Qiu does not disclose or suggest any element of the claimed invention which is missing from Green. Qiu discloses an I α knock-out mouse that produces mouse IgM, IgG and IgA at levels comparable to the wild-type, as discussed above.

Even, if the ordinary skilled artisan were to combine the disclosure of Green with that of Qiu, the ordinary artisan would modify the mouse described by Green by linking the heterologous switch region functionally to an HPRT minigene as taught by Qiu to arrive at a transgenic mammal containing an unrearranged human immunoglobulin heavy chain (IgH) transgene containing V H genes, all the D elements, all J elements, S μ , C μ and C δ from human chromosome 14, and a chimeric human IgH constant region gene in which human constant region gene sequences encoding the desired heavy chain isotype are operably linked to an heterologous switch region which is operably linked to an

HPRT minigene, *i.e.* a transgenic mammal which is even more different from the claimed transgenic mammal than the transgenic mammal of Green.

Furthermore, the prior art and the common knowledge at the time of filing of the present application (*Annex I*), in fact, teach away from the present invention. It is known in the art that B cell maturation, *in vivo*, requires the expression of membrane IgM on B cells surface and that the production of a selected antibody isotype, *in vivo*, requires the development of IgM producing B cells which subsequently undergo isotype class switching from IgM to the IgG or IgA, or IgE isotypes (column 10, lines 43 to 60 of Green and *Annex I*). As discussed above, the mice which are disclosed in Green, Qiu and Harriman (which refer to the same knock-out mouse as Qiu) have IgM producing B cells which subsequently undergo isotype class switching from IgM to the IgG or IgA, or IgE isotype. Therefore, to produce human Ig of the desired isotype (IgG, IgA,) one of ordinary skill in the art would never have engineered mice which do not produce IgM but only Ig of another isotype (IgG, IgA). In accordance with the claimed invention, the present inventors have demonstrated that, in the absence of IgM expression, mammals can surprisingly develop functional B cells able to generate an immune response.

Therefore, even the combined disclosures of the cited references would fail to disclose all the elements of the claimed invention, or suggest this combination or provide a reasonable expectation of success for the present invention because numerous elements from the claimed invention are missing from these reference disclosures. In fact, these references actually teach away from the present invention.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Non-obviousness of claim 58 over Green et al. in view of Qiu et al. and GenBank AC073553.5

Claim 58 stands rejected under 35 USC 103(a) as being unpatentable over Green *et al.* (US 7,547,817), in view of Qiu *et al.* (Int. Immunol., 1999, 11, 37-46) as applied to claims 36-57, 59, and further in view of GenBank AC073553.5.

This rejection is not sustainable over the combination of Green and Qiu for the reasons discussed above. However, GenBank AC073553.5 does not remedy the deficiencies of Green and Qui.

GenBank AC073553.5 discloses the sequence of a 187523 bp DNA segment from mouse chromosome 12.

However, GenBank AC073553.5 does not disclose the specific fragments corresponding to SEQ ID NO: 7 and SEQ ID NO: 8 of the invention, nor the combination of said specific fragments with a human Ca gene or a fragment of said gene comprising the CH3 domain and membrane exons. Furthermore, there is no suggestion of such a combination, nor does this reference provide a reasonable expectation of success the same because the conventional knowledge at the time of filing of the present application actually teaches away from the claimed invention for the reasons discussed above.

Accordingly, this rejection is unsustainable and should be withdrawn.

Non-obviousness of claims 36 and 52 over Green et al. in view of Qiu et al. and Harriman et al.

Claims 36 and 52 stand rejected under 35 USC 103(a) as being unpatentable over Green *et al.* (US 7,547,817), in view of Qiu *et al.* (Int. Immunol., 1999, 11, 37-46) as applied to claims 36-57, 59, and further in view of Harriman *et al.* (J. Clin. Invest., 1996, 97, 477-485).

Harriman was cited as establishing that it was known in the art that the S μ region is not required for IgA class switch, and that a mouse without the genomic S μ region still can produce IgA antibody.

However this is not true for the reasons discussed above. Harriman and Qiu disclose the same I α knock-out mouse. In this knock-out mouse, generated by targeted deletion, the only region of the endogenous IgH locus which is deleted is the I α exon (figure 1 of Harriman). Therefore, all the sequences 5' and 3' to the I α exon which include S μ and S γ in 5' and S α in 3' are present in the I α knock-out mouse IgH locus. Harriman teaches that the I α knock-out mouse produces IgM, IgG and IgA and that the IgA are produced by class switching, *i.e.*, by recombination between the S μ and S α , or S γ and S α regions.

Therefore, Harriman does not disclose or suggest that the S μ region is not required for IgA class switch, and that a mouse without the genomic S μ region still can produce IgA antibody.

Importantly, Harriman fails to disclose all of the elements missing from the two primary references, or suggest this combination or provide any reasonable expectation of success for the present invention. In fact, the combined disclosures of the cited references would teach away from the claimed invention for the reasons noted above.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claims 36-51 and 59 stand rejected under 35 USC 112, first paragraph.

However, the rejection of claims 36-51 and 59 under 35 USC 112, first paragraph, as failing to comply with the written description requirement is respectfully traversed as the IgH locus sequences required for practicing the claimed non-human mammal are described in the specification in such a way as to reasonably convey to one skilled in the

relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The practicing of the claimed invention does not require all the sequences of the IgH locus but only a very small portion of sequences (less than 10 kb) flanking the S μ region, the targeted site for homologous recombination (see figure 2).

These sequences which are necessary and sufficient for practicing the claimed invention are defined by specific features (size, well-known protein domains, *i.e.*, JH/E μ region, C μ gene) which are disclosed in the specification (see figure 2).

The specification provides also the positions of these sequences on mouse chromosome 12 sequence (Genbank/EMBL AC073553).

At the date of filing of the present application, the IgH gene locus had been mapped in different mammal species and its entire sequence or part of its sequence comprising the JH/E μ region and C μ gene sequences were available for numerous species, including at least human, mouse, rat, sheep, cattle, dog, cat, rabbit, hamster, shrew and pig (Schrenzel *et al.*, Immunogenetics, 1997, 45, page 386, 2nd column, end of last paragraph; *Annex II*). Examples of GenBank accession numbers are: AY158087 (bovine), M13800.1 (rat) and X02804.1 (hamster), shown in *Annexes III to V*.

Furthermore, using the mouse sequences that are described in the application, one skilled in the art would have been able to obtain the corresponding sequences from other mammals, using standard molecular biology and/or sequence analysis techniques which were routine in the art at the date of filing of the present application.

Therefore, this ground of rejection is clearly moot.

Claims 36-51 and 59 stand rejected under 35 USC 112, first paragraph.

However, the rejection of claims 36-51 and 59 under 35 USC 112, first paragraph, as failing to comply with the enablement requirement is respectfully traversed as the specification does enable any person skilled in the art to which it pertains, or with it is most nearly connected, to practice the invention commensurate in scope with these claims.

First of all the essential materials (IgH sequences) required for practicing the claimed invention are adequately described by the instant disclosure for the reasons mentioned above.

The specification describes how the claimed transgenic mammal can be obtained by homologous recombination with an appropriate targeting vector (see page 11, lines 4-11, page 11, line 31 to page 12, line 14) and provides a working example of the invention (transgenic mouse line of example 1).

The state of the art and the level of skill in the art is such that pronuclear microinjection of fertilized eggs and the use of *in vitro* embryo production in combination with gene transfer technology are available for the mammal species for which ES cells were not available (Mullins *et al.*, J. Clin. Investigation, 1996, 97, 1557-1560; in particular page 1557, second column, end of paragraph entitled "*Transgenesis by pronuclear injection*").

The inefficiency of pronuclear microinjection due to random integration of the transgene is not a relevant problem for the present invention since the transgene is integrated by homologous recombination in a functional locus. Mullins *et al.* mentions clearly that targeted homologous recombination improves the inefficiency of pronuclear microinjection (see page 1558, first column, middle of first paragraph: "*In many cases, simply including large amounts of flanking sequences may be sufficient to overcome position effects and direct expression to specific tissues*"). Therefore, pronuclear microinjection is not an unpredictable technique in the case of the present invention

since it allows efficient integration of the transgene at the mammal IgH locus and subsequent expression of this transgene.

Furthermore, Mullins *et al.* point out that the use of *in vitro* embryo production in combination with gene transfer technology is a major improvement to pronuclear microinjection of fertilized eggs because transgene screening and cloning can take place before reintroduction into the natural host. Mullins *et al.* mentions that the microinjected embryo technology allows efficient production of transgenic cattle, rabbit and sheep.

Therefore, one skilled in the art with the teaching of the specification and the knowledge in the art would be able to practice the claimed transgenic mammal without undue experimentation.

Therefore, this ground of rejection is deemed to be moot.

Claims 36-51 stand rejected under 35 USC 112, second paragraph.

However, the rejection of claims 36-51 are believed to be obviated by the amendments which were made to claims 36, 46 and 48.

Therefore, withdrawal of the rejection is respectfully requested.

The specification stands objected to.

In view of above amendment to the specification, this ground of rejection is moot.

Finally, Applicants emphasize that even assuming for the sake of argument that one skilled in the art would have had motivation to combine the cited references of record, the artisan still would not have been put in possession of the claimed invention. Moreover, *KSR v. Teleflex*, 550 U.S. 398 (2007), is of no moment to the patentability of the claimed invention since in the present case one skilled in the art could not have

relied upon “common sense” to find implicit motivation to combine the cited references for at least three reasons.

First, even the combined reference disclosures lack the full measure of claimed genetic elements. That is, the combined teachings fall short of the claimed invention.

Second, the combined reference disclosures actually teach away from the claimed invention. Unlike in KSR, where the U.S. Supreme Court found nothing in the prior art that would teach away from the Asano reference (used to determine the obviousness of the claimed invention), on the present record there is cited prior art that teaches away from the claimed invention.

Third, the claimed invention does not constitute a known problem for which there is a known solution. In fact, to the contrary, the cited references strongly indicate that the presently claimed ‘solution’ was anything but known, and, thus patentable.

Guidelines for U.S. Patent Examiners are set forth at MPEP 2143, Eighth Edition (Revision 6, September, 2007). Notably, “Exemplary Rationales” A-G are set forth where an examiner might justifiably cite the KSR decision in support of an obviousness rejection. In fact, none of these rationales apply to the claims at issue. This is striking inasmuch as the KSR test for determining obviousness is clearly broader than the previously (and solely) used ‘teach, suggest, motivate’ (TSM) test. Yet, even an attempted application of the KSR test indicates patentability for the claimed invention based upon the prior art of record.

Annexes I-V are attached to this Amendment in general support of the patentability of the claimed invention. As such, they have not been cited in an Information Disclosure Statement in accordance with standard practice.

Accordingly, in view of all of the above amendments and related remarks, it is believed this application is now in condition for allowance. Favorable consideration and early notice to this effect are earnestly solicited.

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* Annexes I-V were submitted with the Amendment of March 3, 2010.